

AMENDMENTS TO THE SPECIFICATION

In the specification:

Please replace the paragraph at page 6, lines 33-35, with the following paragraph:

~~--Figure 1 shows~~ Figures 1A-1F show the base sequence of cDNA (SEQ ID NO: 1) containing the human UCP-2 promoter region cloned in Example 1 ~~(continued to Figure 2).~~-

Please delete the paragraphs at page 7, lines 1-15.

Please replace the paragraph at page 7, lines 16-17, with the following paragraph:

~~--Figure 7~~ Figure 2 shows the luciferase activity measured in Example 2.--

Please replace the paragraph at page 7, lines 18-19, with the following paragraph:

~~--Figure 8~~ Figure 3 shows the luciferase activity measured in Example 3.--

Please replace the paragraph at page 7, lines 20-23, with the following paragraph:

~~--Figure 9~~ Figure 4 shows the structure of the UCP-2 promoter deficient-clones constructed in Example 4. The numbers in the Figure represent the base number starting from the transcription initiation site.--

Please replace the paragraph at page 7, lines 24-25, with the following paragraph:

~~--Figure 10~~ Figure 5 shows the promoter activity measured in Example 4.--

Please replace the paragraph from page 21, line 32, to page 23, line 18, with the following paragraph:

--Using 0.5 ng of human kidney cDNA (Clontech Laboratory, California, USA) as the template and the base sequence of base number 55 to 82: 5'-ATGGTTGGGTTCAAGGCCACAGATGTGCCC-3' (SEQ ID NO: 2) of previously reported human UCP-2 cDNA [Gimeno, R. et al. (1997), Diabetes, Vol. 46, 900-906] and the base sequence complementary to base number 1300 to 1329: 5'-ATACAGGCCGATGCGGACAGAGGCAAAGCT-3' (SEQ ID NO: 3) as oligonucleotide primers, human UCP-2 gene was amplified by PCR (after heating at 94°C for 5 min., a cycle consisting of heating at 94°C for 1 min, 55°C for 0.5 min and 72°C for 1.5 min was repeated 30 times, followed by heating at 72°C for 5 min), then inserted into pCR-blunt vector. Using this plasmid DNA carrying the insert as the template, oligonucleotide primers were prepared, and probes were prepared using PCR DIG probe synthesis kit (Boehringer-Mannheim Co.) following the attached instruction. Using the prepared probes, human genomic DNA library (Clontech Laboratory, California, USA) in 3×10^6 phages was screened using nitrocellulose filters. Plaque hybridization was performed using DIG Easy hyb (Boehringer-Mannheim Co.), DIG Wash and Block Buffer Set (Boehringer-Mannheim Co.), and DIG nucleic acid detection kit (Boehringer-Mannheim Co.) following the attached instruction. As a result, eight positive clones were obtained from 3×10^6 phages. Of these clones, an inner primer of non-coding exon of previously reported human UCP-2 cDNA sequence [Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687] was synthesized (5'-CAAAGCTGCCAGTGGCTATCATGGCCCG-3') (SEQ ID NO: 4), and a clone containing the non-coding exon was detected by PCR using a primer containing EMBL3 sequence (5'-GACCGGTGCGACCCAGATCTGGGTGCGACCTG-3') (SEQ ID NO: 5), and a genomic clone containing the 5' upstream region of UCP-2 was obtained. From the genomic clone, 3.5 kbp fragment containing UCP-2 promoter region was prepared, and inserted into pCR-blunt vector (Invitrogen Co.), and transformant *E. coli* TOP/10 pCR-UCP2P5' #1-10 was prepared. After that, the restriction enzyme map was prepared and the base sequence was determined. The determined base sequence (SEQ ID NO: 1) is shown in ~~Figures 1 to 6~~ Figures 1A-1F. As shown in ~~Figures 1 to 6~~ Figures 1A-1F, base number 2271 - 2326 and

3416 - 3505 were completely consistent with human UCP-2 cDNA (Gimeno, R.E. et al. (1998), Diabetes, 47 (4), 685-687). Furthermore, the terminal base sequences of the consistent regions were consistent with Shahnborn rule, which is the characteristic of intron-exon boundary base sequence, suggesting that the consistent base sequences are introns. A sequence likely to be CpG island (base number about 2070 - 2270), which is a characteristic of promoters without containing TATA-box sequence, was also confirmed upstream of the first exon. In the promoter sequence described above, PPRE (base number 284 - 296), which is the regulator sequence of promoters of fat cell-related genes, and three C/EBP binding sites (base number 1316 - 1320, 1364 - 1368, 1698 - 1692) were confirmed.--

Please replace the paragraph from page 23, line 29, to page 24, line 4, with the following paragraph:

--EcoRI fragment (3.5 kb) was isolated from the genomic human UCP-2 DNA and blunted using Blunting High Kit (TOYOBO Co.), and then ligated to SmaI-digested pGL3-Basic plasmid DNA. Following the above procedure, human UCP-2 promoter/luciferase vector (pGL3-UCP2) was constructed in which the base number 1 - 3505 shown in ~~Figures 1 to 6~~ Figures 1A-1F was inserted into pGL3-Basic vector. The constructed human UCP-2 promoter/luciferase vector was transiently transfected in HepG2 cells, in which constant expression of UCP-2 was confirmed by RT-PCR, and the activity was examined.--

Please replace the paragraph at page 24, lines 5-24, with the following paragraph:

-- HepG2 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 60,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect Transfection Reagent (QIAGEN Co.), cells were transiently transfected with 1 µg of human UCP-2 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 µg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. Then, the cells were cultured at 37°C in 5% CO₂ for 24 hours, and the luciferase activity was detected using PicaGene Dual Sea Pansy (Nippon Gene Co.) according to the attached instruction. The measurement

data were presented as relative activity to the internal standard value of pRL-SV40-derived sea pansy luciferase activity. The results are shown in ~~Figure 7~~ Figure 2. The human UCP-2 promoter/luciferase vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter. Therefore, the genomic DNA of human UCP-2 gene of this invention has a promoter activity reflecting the in vivo UCP-2 gene expression system.--

Please replace the paragraph from page 24, line 32, to page 25, line 22, with the following paragraph:

--MG-63 cells were seeded in 24-well multiplates (Nunk Co.) at a density of 100,000 cells/well, and cultured at 37°C in 5% CO₂ overnight. Using SuperFect Transfection Reagent (QIAGEN Co.), cells were transiently transfected with 1 µg of human UCP-2 promoter/luciferase vector DNA or pGL3-Basic DNA and 0.1 µg of pRL-SV40 DNA. The procedure was performed according to the attached instruction. The culture medium was exchanged to Dulbecco's modified Eagle's MEM (Gibco Co.) containing 5% rabbit serum (Gibco Co.), and differentiation to fat cell-like cells was induced. Then, the cells were cultured at 37°C in 5% CO₂ for 24, 36, and 72 hours. After culture, the luciferase activity was detected in each culture as described in Example 2. The measurement data were presented as relative activity to the internal standard value of pRL-SV4-derived sea pansy luciferase activity. The results are shown in ~~Figure 8~~ Figure 3. The human UCP-2 promoter/luciferase vector-derived luciferase activity was markedly higher than that of pGL3-Basic lacking the promoter in fat cell-like cells differentiated from human MG-63 cells. Therefore, the genomic DNA of human UCP-2 gene of this invention has the promoter activity reflecting the in vivo UCP-2 gene expression system in fat cell-like cells differentiated from human MG-63 cells.--

Please replace the paragraph from page 25, line 26, to page 26, line 12, with the following paragraph:

--The human UCP-2 promoter/luciferase vector prepared in Example 2 was digested with KpnI and MluI, and the human UCP-2 promoter-deficient vector shown in ~~Figure 9~~ Figure 4 was prepared using Deletion Kit for Kilo-Sequence (Takara Shuzo Co.) following the protocol. The plasmid digested with KpnI and MluI was purified by phenol extraction and ethanol precipitation. Then, the precipitated DNA was treated with exonuclease III and sampled every one minute, and the reaction was terminated. The samples were treated with Mung bean nuclease and the ends were blunted. The ends were restored by Klenow fragment, and the DNA was circularized by DNA ligase. The circularized DNAs were re-treated with MluI to linearize plasmid in which deletion did not occur. *E. coli* JM 109 competent cells (Takara Shuzo Co.) were transformed with this reaction solution. The obtained deficient clone plasmids were purified by publicly known method. The molecular weights of the deficient plasmids were confirmed by agarose gel electrophoresis, and clones were selected. The base sequences of these clones were confirmed by publicly known method.--

Please replace the paragraph at page 26, lines 13-15, with the following paragraph:

--Using these plasmids, the promoter activity was measured by the procedure described in Example 2-~~(Figure 10)~~ (Figure 5).--

The Sequence Listing (both paper and electronic copies) was submitted with the application as filed. If the Sequence Listing has not already been entered, the Examiner is requested to enter it at page 27, line 7, and to renumber the pages with the claims and the abstract accordingly.